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# Quantitative Real-Time PCR

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An abbreviated version of this protocol was published in eLIFE in Feb 2019

Schnyder corneal dystrophy-associated UBIAD1 inhibits ER-associated degradation of HMG CoA reductase in mice

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## Detailed protocol

The original protocol is from 2002 JBC paper "Diminished Hepatic Response to Fasting/Refeeding and Liver X Receptor Agonists in Mice with Selective Deficiency of Sterol Regulatory Element-binding Protein-1c".
But I changed some of the company name or their catalog number here if there were any changes.

Quantitative Real Time PCR—Total RNA was prepared from mouse tissues using an RNA STAT-60 kit (TEL-TEST “B”, Friendswood, TX). Equal amounts of RNA from 3–5 mice were pooled and treated with DNase I (DNA-freeTM; Ambion, Inc.). First strand cDNA was synthesized from 2 ug of DNase I-treated total RNA with random hexamer primers using the cDNA synthesis kit (catalog no. N808-0234; Applied Biosystems). Specific primers for each gene (Table I and Ref. 16) were designed using Primer Express software (Applied Biosystems). The real time PCR contained, in a final volume of 20 ul, 20 ng of reverse transcribed total RNA, 167 nM forward and reverse primers, and 10 ul of 2x Power SYBR Green PCR Master Mix (catalog no. 4367659; Applied Biosystems). PCR was carried out in 384-well plates using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). All reactions were done in triplicate. The relative amount of all mRNAs was calculated using the comparative CT method (41). Cyclophilin mRNA was used as the invariant control for all studies except for liver in which apoB mRNA was used as the invariant control.

**How to cite:**（Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used）

1. Jo, Y. (2020). Quantitative Real-Time PCR. Bio-protocol Preprint. bio-protocol.org/prep442.
2. Jo, Y., Hamilton, J. S., Hwang, S., Garland, K., Smith, G. A., Su, S., Fuentes, I., Neelam, S., Thompson, B. M., McDonald, J. G. and DeBose-Boyd, R. A.(2019). Schnyder corneal dystrophy-associated UBIAD1 inhibits ER-associated degradation of HMG CoA reductase in mice. eLIFE. DOI: 10.7554/eLife.44396

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